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Extraction and Characterization of Pectin from Lanzones (*Lansium domesticum*) Fruit Peel

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Abstract

The peel of Lanzones (Lansium domesticum) is an underutilized waste generated from the consumption of Lanzones fruits in the Philippines, yet it holds untapped potential for manufacturing other products rather than being discarded. Pectin was extracted from Lanzones peels using four different extraction conditions involving acid hydrolysis: (2 pH, 75°C), (2 pH, 95°C), (3 pH, 75°C), and (3 pH, 95°C). After the completion of all the extraction procedures, the pectin yield was then calculated, resulting in 18.15%, 10.42%, 3.31%, and 4.72%, respectively. After the extraction, the pectin was stored together and used to evaluate and differentiate its properties, utilizing titration. The equivalent weight averaged 698.41 g/mol, and anhydroUronic acid content averaged 37.55%. Based on the degree of esterification (32.83%) and Methoxyl content (2.15%), the pectin was classified as low Methoxyl pectin, and the moisture content averaged 10.23%, within IPPA standards. The extraction involving different conditions displayed a significant difference in the amount of pectin yielded, with 2 pH and 75°C being the highest, averaging 18.15%. The pectin extracted from Lanzones (Lansium domesticum) demonstrated quality attributes consistent with industry standards, except for the measured Anhydrouronic acid content, which fell below the conventional threshold of 65% required for pectin utilization.

Keywords: Pectin, Lanzones peels, Acid hydrolysis, Methoxyl content, Anydrouronic acid

1. Introduction

Pectin is a type of water-soluble carbohydrate found in the cell walls and tissues of plants. In plant fruits, it holds adjacent cell walls together (Tikkanen, 2007). It is a polysaccharide that forms a gel when heated with water and sugar. It is commonly used as a thickener and stabilizer in jams, jellies, and preserves. Pectin can also improve the texture and shelf life of dairy products, such as flavored milk and drinkable yogurt. Pectin is mainly extracted from apples and citrus peels, which are rich in this fiber. However, other sources of pectin include berries, grapes, carrots, sunflower seeds, and soybeans. Different methods of extraction can affect the quality and yield of pectin, such as acid hydrolysis, microwave extraction, and enzymatic extraction (Shoemaker, 2019).

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The Lanzones (Lansium domesticum) fruit is oval, ovoid-oblong, or nearly round, measuring 2.5-5 cm in diameter. This skin is characterized by its light greyish-yellow to pale brownish or pink color and a velvety texture. It can be leathery, thin, or thick, and is notable for containing milky latex (Orwa et al., 2009). It is primarily found in Southeast Asian countries such as Thailand, Malaysia, Indonesia, and the Philippines, and has a rich history of traditional medicinal uses. It has been employed to treat various conditions, including eye inflammation, ulcers, diarrhea, fever, and more. Additionally, it serves as a mosquito repellent, skin moisturizer, and whitening agent (Abdallah, Mohamed, & Ibrahim, 2022).

Highlighting its characteristics and complementing the existing traditional medicinal uses, this study introduced a new source of pectin from Lansium domesticum peels and diversified the raw materials for pectin production in providing broader choices for manufacturers and lessening supply chain vulnerabilities while complementing other pectin research involving local ingredients such as mango peels, and banana peels, as conducted by Gragasin et al. (2012) and Israel et al. (2015) respectively. Not only that but introducing new material may lessen Lanzones' peel waste and prompt future researchers to study other overlooked sources for extracting pectin or other related products.

Specifically, this study aimed to answer the following research questions:

1. How much pectin can be extracted from Lanzones (Lansium domesticum) peels?
2. Is there a significant difference in the amount of extracted pectin based on the acid hydrolysis extraction techniques, namely 2pH at 75°C, 2pH at 95°C, 3pH at 75°C, and 3pH at 95°C?
3. How can the quality of pectin from Lanzones peels be described in terms of:
    a. equivalent weight?
    b. Methoxyl content?
    c. anhydroUronic acid content?
    d. degree of esterification?
    e. moisture content?

2. Methods

Research Design

The experimental procedure conducted by the researchers consisted of two phases, extraction of pectin, based on the studies of Gragasin et. al (2014) and Castillo-Israel et. al (2014), and the measurement and characterization of the pectin's properties, based on the study of Hamid et. al (2022).

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Phase 1: Extraction of Pectin

Materials

For the equipment used in the extraction of pectin, the researchers used a blender, a digital weighing scale, a digital pH meter, a drying oven, a food thermometer, a mortar and pestle, a pair of PVC rubber chemical gloves, 2 (200ml) beakers, 2 glass stirring rods, 2 large resealable bags, 2 portable stoves, 2 pots, 2 spatulas, 12 small resealable bags, 36 pieces of 12.5cm laboratory filter paper, and 48 pieces of disposable nitrile gloves. For the ingredients, the researchers obtained 600 grams of Lanzones peels from 3.25 kilograms of Lanzones bought from the local market. Additionally, 150 grams of citric acid, 5 liters of distilled water, and 1.2 liters of 95% ethanol was used.

Data Collection Procedure

Preparation of Lanzones Peel Powder

The peels were thoroughly washed and dried with cloth, then placed in an oven and dried for 5 hours at 60°C. Once dried, the peels were cooled to room temperature. Subsequently, the dried peels were powdered using a blender, and the powdered fruit peels were placed in a sealed bag for later use.

Extraction of pectin from Lanzones peels

The powdered Lanzones peels were separated into four groups for different treatments. One batch utilized distilled water and citric acid to achieve a pH of 2, while another batch aimed for a pH of 3 using the same components. Both batches were subjected to varying temperatures, specifically 75°C and 95°C, and underwent a 60-minute heating process. This experimental design was implemented to determine the most effective technique for pectin extraction using acid hydrolysis. Table 1 below summarizes the experimental plan:

Table 1

Treatment	pH level	Temperature (°C)
Batch 1	2	75
Batch 2	2	95
Batch 3	3	75
Batch 4	3	95

The resulting extract was cooled to 60°C, and its solution was then filtered through a layer of filter paper with a funnel to obtain a liquid called "pectin liquor". Subsequently, twice the volume of 95% ethanol was added to the collected filtrate or "pectin liquor". The solution was allowed to sit overnight for complete precipitation. Afterward, the solution was passed through a layer of filter paper with a funnel to separate the wet pectin from the solution. Once separated, the wet pectin was dried for 6 hours at 60°C. Following drying, the dried pectin

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extract was weighed and then crushed into a fine powder. The powdered pectin was placed in a sealed bag for later use. Finally, the percent recovery of pectin was calculated by dividing the weight of the produced pectin by the weight of the dried Lanzones peel and multiplying by 100%. This provided a measure of the efficiency of the extraction process. Figure 1 below summarizes the extraction process:

Figure 1

Phase 2: Measurement and Characterization of Properties

Materials

For the equipment used in the measurement and characterization of the extracted pectin, the researchers used a drying oven, a digital weighing scale, a 250 ml Erlenmeyer flask, a 500 ml Erlenmeyer flask, a 10 ml glass pipette, a glass stirring rod, 2 (200 ml) beakers, and 3 petri dishes. As for the ingredients, 1 liter of distilled water, 15 ml of 95% ethanol, 5.2 ml of Muriatic Acid (28% HCL), 18 ml of phenolphthalein indicator solution, 1.5 grams of dry pectin, 3.5 grams of sodium hydroxide, and 3 grams of sodium chloride (table salt) was used.

Data Collection Procedure

Preparation of Chemicals: 0.1N and 0.25N NaOH, and 0.25N HCL

The quantity of Sodium Hydroxide (NaOH) required to prepare a 250 milliliter 0.1N solution was calculated by multiplying the normality desired with the equivalent weight and the volume in liters (Bashyal, 2023).

Grams of compound needed = (N desired) x (equivalent mass) x (volume in liters)

Grams of compound needed = (0.1) x (40) x (0.25) = 1 ml/g

A digital weighing scale was used to measure precisely 1 gram of sodium hydroxide (NaOH). This was then added to 125 milliliters of distilled water in an Erlenmeyer flask. A stirring rod was then used to dissolve the NaOH in the water. Subsequently, the solution was cooled down to an ambient temperature. Finally, distilled water was added to the solution, and adjusted the volume to 250 milliliters. The solution was stirred thoroughly to ensure effective mixing.

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The quantity of Sodium Hydroxide (NaOH) required to prepare a 250 milliliter 0.25N solution was calculated by multiplying the normality desired with the equivalent weight and the volume in liters (Bashyal, 2023).

Grams of compound needed = (N desired) x (equivalent mass) x (volume in liters)

Grams of compound needed = (0.25) x (40) x (0.25) = 2.5 ml/g

Two and a half grams of sodium hydroxide (NaOH) was added to 125 milliliters of distilled water in an Erlenmeyer flask, then the NaOH was dissolved into the water with a stirrer. The solution was then allowed to cool to room temperature. Distilled water was then added to the solution to adjust the volume to 250 milliliters. The solution was then thoroughly stirred.

The quantity of muriatic acid (28% HCl) required to prepare a 200 milliliter 0.25N HCl solution was calculated by multiplying the normality desired with the equivalent weight and the volume in liters desired to get the grams of the compound required, then dividing the product by the percent concentration and the specific gravity (Bashyal, 2023).

Grams of compound required = (N desired) x (equivalent mass) x (volume in liters desired)

Volume of concentrated acid required = $grams\ of\ acid\ needed$ ($percent\ concentration$*$specific\ gravity$)

Grams of compound required = (0.25) x (36.5) x (.2) = 1.825

Volume of concentrated acid required = $\frac{1.825}{.28*1.18} = 5.5236$ ml/g

Twenty-five milliliters of distilled water were poured into a 250 milliliter Erlenmeyer flask along with 5.5236 milliliters (about 5.5 milliliters) of 28% concentrated HCl. One hundred fifty milliliters of water was added after, then the solution was allowed to cool to room temperature. More distilled water was added until the volume of the solution reached 250 milliliters. The solution was then stirred thoroughly to ensure effective mixing.

Determination of equivalent weight

One half gram of Lansium domesticum pectin was mixed with five milliliters of ethanol, one gram of NaCl, one hundred milliliters of distilled water, and six drops of Phenolphthalein until the pectin was fully dissolved. The solution was then transferred into an Erlenmeyer flask and titrated with 0.1N NaOH until the solution turned pink. During titration, 0.5ml of the titrant was added using a pipette every 5 seconds. The equivalent weight was calculated by multiplying the weight of the sample in grams by 1000 and then dividing by the volume of NaOH in milliliters multiplied by the normality of NaOH.

$$\text{Equivalent Weight} = \frac{1000*Weight\ of\ the\ sample\ (g)}{volume\ of\ NaOH*normality\ of\ NaOH}$$

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Determination of Methoxyl content

Twenty-five milliliters of 0.25N NaOH were added to the solution obtained in the equivalent weight process, and the mixture was allowed to sit at room temperature for 30 minutes. Subsequently, twenty-five milliliters of 0.25N HCl were added to the solution, which was then titrated using 0.1N NaOH until it turned pink. During titration, 0.5ml of the titrant was added using a pipette every 5 seconds.

The Methoxyl content was calculated by multiplying the volume of NaOH in milliliters by the normality of NaOH and 31 then dividing by the weight of the sample in milligrams.

$$\text{Meo\%} = \frac{Volume\ of\ NaOH\ (mL) * Normality\ of\ NaOH * 31}{Weight\ of\ the\ sample\ (mg)}$$

Determination of Anhydrouronic acid content

The Anhydrouronic acid content was calculated by multiplying the molecular weight of anhydrouronic acid by the normality and the sum of the sodium hydroxide used in the determination of equivalent weight content in milliliters and the sodium hydroxide used in the determination of Methoxyl content in milliliters. This sum was then divided by the weight of the sample in grams multiplied by 1000, and the quotient was multiplied by 100.

$$\text{AU A\%} = \frac{[176 * 0.1(N)(mL)] + \{V1(mL) + V2(mL)\}}{Weight\ of\ the\ sample\ (g) * 1000}\ x\ 100$$

*176 is the molecular weight of Anhydrouronic acid. V1 is the amount of sodium hydroxide used in determination of equivalent weight content and V2 is the amount of sodium hydroxide used in the determination of Methoxyl content.

Determination of degree of esterification

The degree of esterification was calculated by multiplying the formula weight of Anhydrouronic acid by the Methoxyl content, divided by the formula weight of Methoxyl that was multiplied to the Anhydrouronic acid content. This quotient was then multiplied by 100.

$$\text{DE\%} = \frac{176 * MEO\%}{31 * AUA\%}\ x\ 100$$

*176 and 31 are the formula weights of Anhydrouronic acid and MeO respectively.

Determination of moisture content

One gram of pectin was placed in a container and heated at 130°C for 2 hours, after which it was weighed. The moisture content was calculated by subtracting the final weight in grams from the initial weight in grams and dividing by the initial weight in grams. The resulting quotient was then multiplied by 100.

$$\text{Moisture Content \%} = \frac{Initial\ weight\ (g) - Final\ weight\ (g)}{Initial\ weight\ (g)}\ x\ 100$$

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Data Analysis

Conversion of Pectin Yield to Percentage

The raw data for pectin yield was converted from grams to percentage to ensure a more accurate representation. This conversion was achieved using the following formula:

$$\text{Pectin Yield \%} = \frac{Amount\ of\ pectin\ (g)}{Dried\ peel\ weight\ (g)}\ x\ 100$$

This formula accounts for the amount of pectin obtained relative to the weight of the dried peel, providing a standardized measure for comparison.

Statistical Analysis: Two-way ANOVA

The data for pectin yield was subjected to a two-way Analysis of Variance (ANOVA) to assess the effects of two categorical factors on the pectin yield. This statistical test helps determine whether there are significant differences in pectin yield across different levels of each factor and whether there are interactions between the factors.

Tukey's Post-hoc Analysis

Upon detecting significant differences in pectin yield between groups or conditions in the ANOVA, Tukey's post-hoc analysis was conducted to identify specific pairwise differences. This analysis helps to determine which groups differ significantly from each other.

Calculation of Pectin Properties

The properties of the extracted pectin were analyzed for their mean and standard deviation. This involved calculating the average value for each property across all samples and determining the degree of variability around the mean using the standard deviation.

3. Results

Table 2

Amount of Pectin Extracted in Grams(g)

Conditions	Dried Peel Weight	Amount of Pectin
2pH 75°C		
First Trial	9	1.5
Second Trial	9	1.9
Third Trial	9	1.5
Average	9	1.63
2pH 95°C		
First Trial	12.5	1.2
Second Trial	12	1.3
Third Trial	12	1.3
Average	12.17	1.27

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3pH 75°C		
First Trial	12.1	0.5
Second Trial	12.1	0.3
Third Trial	12.1	0.4
Average	12.1	0.4
3pH 95° C		
First Trial	12	0.5
Second Trial	12	0.6
Third Trial	12	0.6
Average	12	0.57

As seen in Table 2, among the four conditions, Group B (2pH 75°C) yielded the most amount of pectin, averaging 1.63g, followed by Group A (2pH 95°C) averaging 1.27g, Group C (3pH 95°C) averaging 0.57g, and Group D (3pH 75°C) with the least amount of pectin, averaging 0.4g. The group maintained the amount of dried peel used for the extraction near 12 grams, except for Group B, which used 9 grams instead of the planned 12 grams. This adjustment was made due to the lack of dried Lanzones peels left and available Lanzones during the experimentation period. Additionally, Group B's trial was executed last. This demonstrates that the exact amount of pectin in grams cannot be the basis for further analysis, as the weight of dried peel used varies.

Table 3

Pectin Yield (%)

	2pH 75°C	**2pH 95°C**	**3pH 75°C**	**3pH 95°C**
First Trial	16.67	9.6	4.13	4.17
Second Trial	21.11	10.83	2.48	5
Third Trial	16.67	10.83	3.31	5
Average	18.15	10.42	3.31	4.72

Table 3 shows the yield calculated based on the percentage of the amount of the extracted pectin following the amount of the dried peel it was extracted from. Group B (2pH 75°C) had the highest pectin yield with an average of 18.15%, followed by Group A (2pH 95°C) averaging 10.42%, Group C (3pH 95°C) averaging 4.72%, and Group D (3pH 75°C) with the least yield, averaging 3.31%.

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Table 4

Results of Two-Way ANOVA

Cases	Sum of Squares	df	Mean	F	P
pH Level	16.67	1	316.419	158.491	< .001
Temperature	29.89	1	29.894	14.973	0.005
pH Level * Temperature	62.746	1	62.746	31.429	< .001
Residuals	15.972	8	1.996		

Table 4 presents the analysis conducted using Two-Way ANOVA to determine the significant difference in the amount of pectin extracted under the four conditions, considering the two independent variables: pH level and temperature. The analysis based on pH level resulted in a p-value of <0.001, while the analysis based on temperature resulted in a p-value of 0.005. The combined analysis of pH level and temperature yielded a p-value of <0.001, leading the researchers to reject the null hypothesis and proceed with post-hoc analysis.

Table 5

*Post Hoc Test - pH Level*Temperature*

		Mean Difference	SE	t	Ptukey
2pH 75°C	3pH 75°C	14.843	1.154	12.866	< .001***
	2pH 95°C	7.730	1.154	6.7	< .001***
	3pH 95°C	13.427	1.154	11.638	< .001***
3pH 75°C	2pH 95°C	-7.113	1.154	-6.166	0.001**
	3pH 95°C	-1.417	1.154	-1.228	0.628
2pH 95°C	3pH 95°C	5.697	1.154	4.938	0.005***

As indicated in Table 5, due to the presence of significant differences in the amount of extracted pectin, the researchers conducted Tukey's post-hoc analysis. All group pair comparisons resulted in p-values less than 0.05, indicating significant differences, except for the pair of Group D (3 pH 75°C) and Group C (3pH 95°C), which resulted in a p-value of 0.628, indicating no significant difference between the amount of pectin extracted under these two conditions.

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Table 6

Properties of Extracted Pectin

Property	R1	R2	R3	Mean	SD
Equivalent Weight (g/mol)	666.67	714.29	714.29	698.41	27.49
Methoxyl Content (%)	2.17	2.17	2.17	2.17	0
AnhydroUronic Acid (%)	38.72	36.96	36.96	37.55	1.02
Degree of Esterification (%)	31.82	33.33	33.33	32.83	0.87
Moisture Content (%)	12	11.3	7.4	10.23	2.48

Table 6 provides an overview of the different values regarding the properties of the extracted pectin based on equivalent weight, Methoxyl content, Anhydrouronic acid, degree of esterification, and moisture content.

4. Discussion

The observed relationship between pH level, temperature, and pectin yield, as evidenced by the four given conditions in Table 3, hints at a correlation where lower pH levels and temperatures tend to result in higher pectin yields. However, it's important to note that this conclusion is limited to the specific conditions studied, and there may be other unexplored variables and extraction methods relevant to pectin extraction from Lanzones peels. Pérez et al. (2022) emphasize the significance of pH level as the primary determinant of pectin yield, corroborating the notion that lower pH levels contribute to higher yields. Additionally, Nadar, Arora, & Shastri (2022) further elaborate that while higher acid strength generally leads to increased pectin yield, extreme acidity, such as a pH of 1, can result in severe acid hydrolysis and degradation of pectin, highlighting the complexity and nuances involved in optimizing pectin extraction processes.

As shown in Table 6, the different properties of the extracted pectin, affect the quality of the product.

Property 1: Equivalent Weight

Equivalent weight represents the quantity of unesterified Galacturonic acid within the molecular structure of pectin. The presence of non-esterified Galacturonic acid within pectin's molecular chains contributes significantly to its viscosity and water-binding characteristics. The equivalent weight of the extracted pectin is at 698.41 g/mol which is far from that of commercial citrus pectin at 893.00 g/mol (Castillo-Israel et. al, 2015), indicating the extracted pectin has lower gelling capacity. This value is also used to calculate the other properties.

Property 2: Methoxyl Content

Methoxyl content represents the degree of methylation of pectin molecules; it is the ability to disperse in water effectively and establish either hydrogen bonds or bonding through the formation of dimmers using divalent cations (Chandel et. al, 2022). This is crucial for determining the pectin’s suitability for food and pharmaceutical applications. Higher

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Methoxyl content leads to better gelling properties while requiring sugar and acid. Low Methoxyl pectin forms gels in the presence of calcium ions. (Sayed et. al, 2022). This value is also used to calculate the degree of esterification. Based on the calculated degree of esterification, the extracted pectin is considered as low Methoxyl pectin.

Property 3: Anhydrouronic Acid

AUA content reflects the degree of Uronic acid loss during pectin extraction. Higher AUA content correlates with better gelling ability and improved texture in food products. Higher AUA content correlates with the better gelling ability and improved texture in food products (Sayed et. al, 2022). The percentage to accept the use of pectin in food, cosmetics, and pharmaceutical industries must contain a percentage of >65% (Baraiya et. al, 2023). Due to the average amount of AUA in the extracted pectin being 37.55%, it cannot be used for such services or products.

Property 4: Degree of Esterification

The DE represents the proportion of esterified carboxyl groups in pectin; it determines the use of pectin as a gelling and thickening agent. High DE results in rapid gel formation, while low DE pectin requires calcium ions for gelation and takes longer to form (Sayed et. al, 2022). The extracted pectin is considered as low Methoxyl pectin as it has a degree of esterification less than 50%.

Property 5: Moisture Content

Moisture content refers to the water content in pectin. Proper moisture levels help prevent microbial growth, maintain pectin stability during storage, and longer shelf life (Virk & Sogi, 2007). According to the IPPA guidelines, the acceptable amount of moisture content in pectin is ≤ 12%, in which the extracted pectin fulfills by not reaching an amount higher than 12% (IPPA in Susanti et. al, 2021).

5. Conclusion and Recommendation

The amount of pectin extracted from Lanzones peels ranged from 3.31% to 18.15% depending upon the condition. Accounting for this, there is a significant difference in the amount of pectin extracted using the four different conditions: (2 pH, 75°C), (2 pH, 95°C), (3 pH, 75°C), and (3 pH, 95°C). The quality assessment of the pectin underscores several key points crucial for its application in manufacturing processes. The equivalent weight measurement highlights its lower gelling capacity in comparison to commercial citrus pectin, thereby suggesting potential limitations in its functionality for certain product formulations.

Moreover, the Anhydrouronic acid content falls below industry standards, rendering the pectin unsuitable for widespread manufacturing use. However, it's noteworthy that the moisture content aligns with IPPA standards, indicating a favorable aspect of the pectin's

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quality. Additionally, the classification of the pectin as low Methoxyl, based on its degree of esterification and Methoxyl content.

The research group recommends preparing equipment and methods to accurately gather and measure data. In the case of this study, the researchers recommend using more precise weighing equipment and the use of a burette for the titration process, which is unavailable at the school's laboratory during the experimentation, which would have made the results more accurate. Additionally, future researchers may explore other pectin extraction conditions and methods for Lanzones peels or other sources.

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